Automated Circuit Design in Saccharomyces cerevisiae

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Project Goals: Our objective is to develop a new standard for the engineering of microbial systems based on rational design, engineering, and optimization of hybrid regulatory networks. We envision a future biorefinery that is based on the development of designer organisms that have exquisite and predictable control architectures governing the expression of a range of valuable traits. Computer aided design platforms will guide the assembly of synthetic constructs containing orthogonal heterologous circuits to recode native regulatory networks. Together, these will enable predictable and dynamic control of multiple designer phenotypes such as: i) growth on various feedstocks in consolidated bioprocesses, ii) feedback control to mitigate accumulation of toxic metabolites, iii) production of target molecules (C3-C4 alcohols), and/or iv) robustness to process upsets (e.g. temp., phage). The focus of this proposal is to develop the technical and computational infrastructure to enable this vision. We will develop this platform first in the model organism *E. coli* and then in DOE relevant non-model organisms.

Genetic circuits are widely used in scientific, industrial and therapeutic approaches. In prokaryotic systems, genetic circuits are successfully designed and built by Cello, the genetic circuit design automation platform. However, building genetic circuits in eukaryotic systems are challenging due to the limited sensors and regulatory units. Here, we developed a method to systematically import bacterial transcription factors into Saccharomyces cerevisiae to create a bunch of candidate sensors and NOT gates. We next determined strategies to link these elements together with minimum cross interactions. Finally, we incorporated these novel components and construction strategies with Cello to design DNA sequence. By using this platform, we successfully created several large circuit constructions (6-8 gates, 9–11 regulators, up to 20 regulatory operons) in yeast. We further built an ODE model to investigate the dynamics when input states changed. Our model predicted the intermediate faults during several transferences, which were confirmed in the following experiments. In this study, we demonstrate a general approach of circuit design automation in a novel eukaryotic system. This will highly expand our ability to build complicated circuits across different organisms.

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